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L2: Entry 1 of 4

File: USPT

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DOCUMENT-IDENTIFIER: US 6265201 B1

TITLE: DNA molecules and protein displaying improved triazine compound degrading ability

DEPR:

In a preferred method of this invention, gene shuffling, also termed recursive sequence recombination, is used to generate a pool of mutated sequences of the atzA gene. In this method the atzA gene, alone or in combination with the atzB gene, is amplified, such as by PCR, or, alternatively, multiple copies of the gene sequence (atzA and atzB) are isolated and purified. The gene sequence is cut into random fragments using enzymes known in the art, including DNAase I. The fragments are purified and the fragments are incubated with single or double-stranded oligonucleotides where the oligonucleotides comprise an area of identity and an area of heterology to the template gene or gene sequence. The resulting mixture is denatured and incubated with a polymerase to produce annealing of the single-stranded fragments at regions of identity between the single-stranded fragments. Strand elongation results in the formation of a mutagenized double-stranded polynucleotide. These steps are repeated at least once. In this gene shuffling technique, recombination occurs between substantially homologous, but non-identical, sequences of the atzA gene. In the studies provided in Example 2, the atzB gene was not gene-shuffled.

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